PHARMACOKINETICS

Limited sampling strategy for determining metformin area under the plasma concentration–time curve

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AIM

The aim was to develop and validate limited sampling strategy (LSS) models to predict the area under the plasma concentration–time curve (AUC) for metformin.

METHODS

Metformin plasma concentrations ($n = 627$) at 0–24 h after a single 500 mg dose were used for LSS development, based on all subsets linear regression analysis. The LSS-derived AUC(0,24 h) was compared with the parameter 'best estimate' obtained by non-compartmental analysis using all plasma concentration data points. Correlation between the LSS-derived and the best estimated AUC(0,24 h) (r^2), bias and precision of the LSS estimates were quantified. The LSS models were validated in independent cohorts.

RESULTS

A two-point (3 h and 10 h) regression equation with no intercept estimated accurately the individual AUC(0,24 h) in the development cohort: $r^2 = 0.927$, bias (mean, 95% CI) –0.5, −2.7–1.8% and precision 6.3, 4.9–7.7%. The accuracy of the two point LSS model was verified in study cohorts of individuals receiving single 500 or 1000 mg ($r^2 = -0.933–0.934$) or seven 1000 mg daily doses ($r^2 = 0.918$), as well as using data from 16 published studies covering a wide range of metformin doses, demographics, clinical and experimental conditions ($r^2 = 0.976$). The LSS model reproduced previously reported results for effects of polymorphisms in *OCT2* and *MATE1* genes on AUC(0,24 h) and renal clearance of metformin.

CONCLUSIONS

The two point LSS algorithm may be used to assess the systemic exposure to metformin under diverse conditions, with reduced costs of sampling and analysis, and saving time for both subjects and investigators.

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Metformin, the most widely prescribed drug in type 2 diabetes mellitus, shows considerable inter-individual variability in clinical response, prompting a considerable amount of research on its pharmacokinetics.
- Metformin pharmacokinetics is usually analyzed using non-compartmental methods, which require the collection and analysis of multiple blood samples, a costly and time-consuming procedure for patients, clinical and analytical staff.
- Evidence from various therapeutic classes indicates that pharmacokinetic parameters, such as the area under the plasma concentration *vs.* time curve (AUC), can be accurately estimated using limited sampling strategies (LSS). coupled to regression analyses.

WHAT THIS STUDY ADDS

- Two-point (3 h and 10 h) regression equations estimated accurately the individual AUC in the 24 h following the administration of single (500–1000 mg) or repeated doses (1000 mg) of metformin to Danish and Brazilian healthy individuals, studied by our groups.
- The accuracy of the two point LSS model was verified using data from 16 published studies, covering a wide range of metformin doses, demographics, clinical and experimental conditions, including pharmacogenomic variables.
- We conclude that the two point LSS algorithm may be used to assess the systemic exposure to metformin under diverse conditions, with reduced costs of sampling and analysis, and saving time for both subjects and investigators.

Introduction

Metformin, a biguanide derivate, is the most widely prescribed drug to manage glucose metabolism in individuals with type 2 diabetes mellitus (T2DM), and is recommended as a first line medication in the joint guidelines of the American Diabetes Association and the European Association of the Study of Diabetes [1]. The drug has a favourable safety/risk profile, and in addition to lowering blood glucose level, metformin may have positive effects on diabetes related endpoints, including macrovascular and microvascular events [2]. Nevertheless, there is considerable inter-individual variability in response to metformin and it has been shown that its use as monotherapy may not provide adequate control in 15–30% T2DM patients [3, 4]. This has prompted a considerable amount of research on metformin pharmacokinetics, including drug-drug interaction [5] and pharmacogenetic studies [6–8].

Metformin pharmacokinetics are usually analyzed using non-compartmental methods, which require the collection and analysis of multiple blood samples, a costly and time-consuming procedure for patients, clinical and analytical staff. Ratain *et al.* [9, 10] and Egorin *et al.* [11] were the first to demonstrate that pharmacokinetic parameter measurements could be simplified by using a technique called limited sampling strategy (LSS). They showed that regression equations using three blood samples collected at specific times provided accurate estimates of the area under the plasma concentration *vs.* time curve (AUC) of the anticancer drugs, vinblastine, amonafide and cyclophosphamide. The LSS technique has been successfully applied to several other therapeutic groups, such as immunosuppressants [12], antifungal [13], antibiotics [14], antiviral [15], corticosteroids [16] and calcium antagonists [17]. In the present study we explored the application of LSS to assess the systemic exposure to metformin, using the AUC in the 24 h following drug administration (AUC(0,24 h)) as the pharmacokinetic parameter.

Methods

Development of LSS models for the AUC of metformin

Metformin plasma concentrations ($n = 627$) from 50 healthy, adult Danish individuals enrolled in a previously published study [18] were used for the development of LSS models to estimate the AUC(0,24 h) following oral administration of a single 500 mg dose of metformin (Orabet® Hexal, Hvidovre, Denmark). WinNonlin 6.3 (Pharsight, Mountain View, CA,

USA) was used to perform non-compartmental analysis of metformin plasma concentrations and linear trapezoidal interpolation was used to determine AUC(0,24 h). The AUC (0,24 h) thus obtained is taken as the 'best estimate' of the parameter value (see below). All subsets linear regression analysis [19] of the AUC(0,24 h) best estimates against the metformin plasma concentration at a particular time (C_{time}) was carried out in order to develop LSS models. Computations were carried out using function leaps [20] in Splus 4.0 [21]. This analysis produced equations of the following form: AUC(0,24 h) = $Ao + A1*C1 + A2*C2 . . . + An* Cn$, where An are coefficients and there is a variable number of samples. Regression equations were ranked according to the r^2 criteria in order to identify those that provided the best fit for 1 to 10 timed plasma samples. The LSS-predicted AUC(0,24 h) were then compared with the AUC(0,24 h) best estimates for each of the 50 individuals. The bias of these LSS-derived estimates was assessed by calculating the mean percentage of difference (MD%) from the best estimates as follows: MD% = [(derived estimate – best estimate)/best estimate]*100%. Precision was assessed by calculating the mean absolute percentage of difference (MAD%) as follows: MAD% = [(|derived estimate – best estimate|)/best estimate]*100 [13, 14]. Following suggestions of two referees who evaluated the original manuscript, we constructed Blant–Altman plots to visualize the agreement between best-estimated and LSS-predicted AUC (0,24 h)s and applied Loess models to fit smooth surfaces to the correlation between the two AUC(0,24 h) values.

Validation of the LSS model for the AUC of metformin

The LSS models developed for estimating the AUC(0,24 h) of metformin were validated in various data sets by applying the regression equations derived in the development cohort (above) to the concentrations observed at the same respective times, but under different experimental conditions. The AUC (0,24 h)'s thus obtained (LSS-predicted AUC(0,24 h)) were then compared with the best estimates of this metric. Three data sets were used for validation, namely:

1 Two-hundred and fifty-six adult Brazilians enrolled in nine bioequivalence trials, performed according to the guidelines of ANVISA, the Brazilian Health Surveillance Agency (www.portal.anvisa.gov.br) and approved by the respective IRBs. Each volunteer (24–34 per trial) provided written, informed consent. The bioequivalence trials adopted an open-label, randomized, two sequence, two period crossover design, in which administration of the reference (Glifage®, Merck & Co., Rio de Janeiro, Brazil) and a test

metformin formulation were separated by a 7 day washout interval. The oral metformin doses were 500 mg in four trials (113 volunteers, n = 1640 samples) and 1000 mg in five trials (143 volunteers, n = 2037 samples). Consecutive blood samples were collected during the 24 h following metformin administration, the concentration of metformin in plasma was determined by LC/MS/MS and a non-compartmental model provided by WinNonlin software was used to calculate the AUC(0,24 h) best estimates. Only data for the reference metformin formulations (500 or 1000 mg) were used for LSS validation.

2 Nineteen healthy, adult Danish individuals enrolled in a drug–herb interaction study between metformin and St John's wort [22] in which each subject received daily oral doses of metformin for 7 days in escalating doses with a target of 1000 mg twice daily. Details of the study protocol are described in the published report [22]. The plasma metformin concentration data (n = 266) and the AUC(0,24 h) at day 7 were used for validation of the LSS equations.

3 Metformin plasma concentrations (n = 412) were gathered from 16 previously published studies [23–28] conducted under a variety of experimental conditions. Scanned plots of the published plasma concentrations *vs.* time curves were used to obtain the plasma concentration data points and to calculate the AUC(0,24 h) best estimates, by means of the trapezoidal method.

Applying the LSS model to a pharmacogenetic trial

The LSS model was applied to examine the association of the *OCT2* c.808G > T (rs316019) genotypes with metformin AUC (0,24 h), using previously published data from one of our groups [18]. Data from 46 of the 50 individuals were available for the LSS analyses. Four individuals were excluded due to missing blood samples at either 3 h or 10 h. Using the estimated AUC from the LSS model we reevaluated statistical inference of the gene–gene interaction between *OCT2* c.808 (G > T) and *MATE1* g.-66 T > C on the renal clearance of metformin.

Results

Development of limited sampling models for AUC(0,24 h)

Figure 1A shows the plasma metformin concentration–time curve for the single 500 mg oral dose, used to develop LSS models for the AUC(0,24 h). Best estimates of AUC(0,24 h) ranged from 2.9–13.8 µg l^{-1} h (median = 7.2 µg l^{-1} h). The all-subsets regression approach used to identify the most informative sampling times (Methods) showed that two point (3 h and 10 h) LSS equations, with or without an intercept term, provided accurate estimates of the individual AUC (0,24 h) (Table 1). The individual AUC(0,24 h) derived from these two point LSS equations correlated closely (r^2 = 0.927, mean bias 0.5–0.7%, mean precision 6.2–6.3%) with the AUC(0,24 h) best estimates. Figure 1B shows a scatter plot of the best estimated *vs.* the LSS-predicted AUC(0,24 h) by both two point equations. Blant–Altman and Loess plots of these data are shown in Supplementary Figures S1 and S2, respectively. Increasing the number of sampling points to more than two increased r^2 marginally and added little to the bias and precision of the estimates (data not shown). Based on the statistical principle of parsimony, we settled for the two point regression without an intercept term for validation in different data sets.
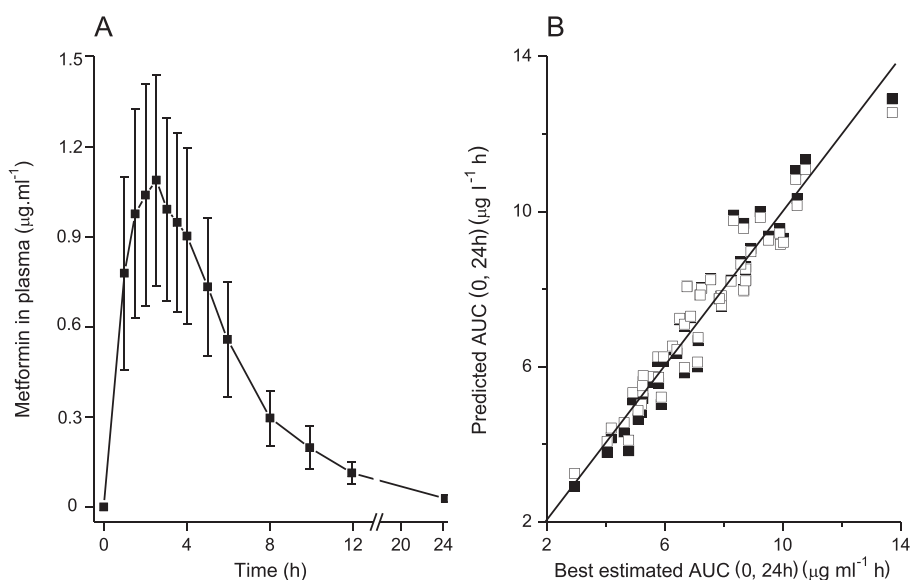

Figure 1

A. Mean (± s.d. of the mean) concentrations of metformin in the plasma of healthy Danes, after a single 500 mg oral dose. B. Scatter plot of the relationship between the individual best estimated AUC(0,24 h) and the corresponding AUC(0,24 h) derived from the two point (3 h, 10 h) LSS models, with (closed squares) or without (open squares) intercept. The LSS equations are shown in Table 1. The continuous line is the identity line

Table 1

r^2, bias and precision of the most informative linear equations for two sample times (3 h, 10 h) to estimate the AUC(0,24 h) of metformin

Cohorts	LSS equations	r^2	MD (%) mean (95% CI)	MAD (%) mean (95% CI)
Development cohort				
Healthy Danes, single dose, 500 mg	$4.779*C_3 + 13.174*C_{10}$	0.927	−0.5 (−2.7, 1.8)	6.3 (4.9, 7.7)
	$0.543 + 4.454*C_3 + 12.273*C_{10}$	0.927	0.7 (−1.4, 2.8)	6.2 (4.9, 7.5)
Validation cohorts				
Healthy Brazilians, single dose, 500 mg	$4.779*C_3 + 13.174*C_{10}$	0.934	3.9 (2.2, 5.5)	7.9 (6.9, 8.9)
Healthy Brazilians, single dose, 1000 mg	$4.779*C_3 + 13.174*C_{10}$	0.933	1.0 (−0.3, 2.3)	7.2 (6.3, 8.1)
Healthy Danes, repeated doses, 1000 mg	$4.779*C_3 + 13.174*C_{10}$	0.918	−4.4 (−7.4, 1.5)	6.8 (5.2, 8.5)
Published data	$4.779*C_3 + 13.174*C_{10}$	0.976	−1.0 (−2.7, 0.7)	4.3 (3.3, 5.3)

r^2, correlation coefficient between LSS-predicted and best-estimated AUC$_{0-24h}$; MD, bias; MAD, precision of the LSS-predicted estimates. Published data: [23–38]

Validation of limited sampling models for AUC (0,24 h)

In the first validation exercise, the two point LSS equation developed in the Danish cohort was applied to data from 113 healthy Brazilians enrolled in bioequivalence trials, in which they were given a single 500 mg dose of the reference metformin formulation. Best estimates of AUC(0,24 h) in this set ranged from 1.9–10.3 µg l^{-1} h (median = 4.8 µg l^{-1} h). The results, shown in Table 1, Figure 2A and Supplementary Figures S3 and S4 indicate that the two point LSS equation provided accurate estimates of the AUC(0,24 h) (r^2 = 0.934,

bias 3.9%, precision 8.9%). A similar performance (r^2 = 0.933, bias 1.0%, precision, 8.4%, Table 1) was obtained when the two point LSS model was applied to another set of 137 healthy Brazilians, who had been given a single 1000 mg dose of the reference metformin formulation in bioequivalence trials. The best estimates of AUC(0,24 h) in this set ranged from 3.2–19.8 µg l^{-1} h (median 8.6 µg l^{-1} h). A scatter plot of the best estimated vs. LSS-derived AUC(0,24 h) is shown in Figure 2B. Blant–Altman and Loess plots of these data are presented in Supplementary Figures 5 and 6, respectively.
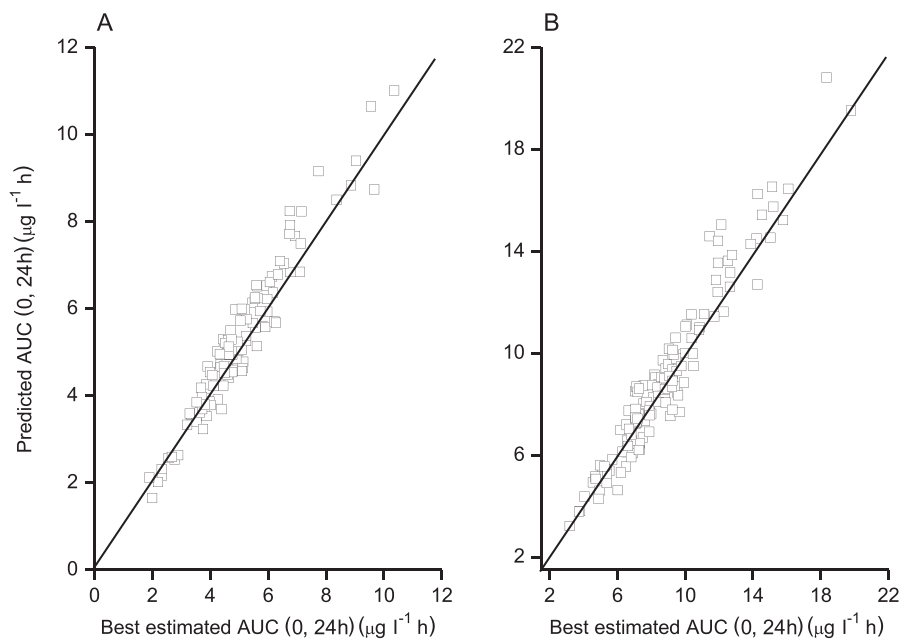
Figure 2

Scatter plots of the relationship between the individual best estimated AUC$_{0-24h}$ and the corresponding AUC(0,24 h) derived from the two oint (3 h, 10 h, no intercept; Table 1) LSS model in healthy Brazilians, after single 500 mg (A) or 1000 mg (B) metformin doses. The continuous line is the identity line

As a second validation approach we applied the two point LSS model to data obtained after seven daily administrations of metformin to healthy Danish individuals, and obtained good estimates of the metformin AUC(0,24 h) ($r^2 = 0.918$, bias −0.44%, precision 6.8%, Table 1). These results suggest that the two point LSS model is capable of providing accurate estimates of metformin AUC(0,24 h) during pharmacokinetic steady-state conditions and, therefore, may be useful for estimation of the systemic drug exposure in patients under chronic treatment with metformin.

The LSS model was further validated using data from 16 previously published studies [23–38]. The results (Table 1, Figure 3, Supplementary Figures S7 and S8) indicated that the AUC (0,24 h) predicted by the two point LSS model was in excellent agreement ($r^2 = 0.976$, bias −1.0%, precision 4.3%) with the corresponding AUC(0,24 h) best estimates, over a wide range (3.8–22.4 µg h l^{-1}) of AUC(0,24 h) values and under a variety of demographic, experimental and clinical conditions. These include male and female gender, various ethnicities (white European and North American, African, Hispanic/Latin, Native and Paci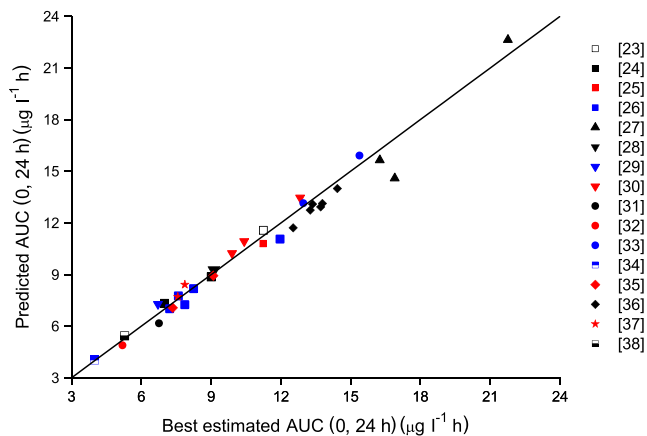fic Islander from United States, Mexican, Chinese, Korean and Japanese) healthy volunteers and patients with pre-existing diabetes, gestational diabetes, polycystic ovarian syndrome or post-gastric bypass surgery, as well as different genotypes at various loci in drug transporter genes. Different metformin formulations (immediate or extended release tablets and liquid formulations), administration regimens (single, two or repeated doses) and a wide range of doses (250–3000 mg day^{-1}) were used in these studies.

Finally, we explored the potential utility of the LSS in pharmacogenetic studies, using published data from Christensen et al. [18] on the influence of polymorphisms in the OCT2 and MATE1 genes on metformin pharmacokinetics. As shown in Table 2, almost similar P values were obtained for the association between OCT2 c.808G > T genotypes and the metformin AUC(0,24 h) when the AUC(0,24 h) was estimated using all data points (as reported by Christensen et al. [18], $P = 0.49$) or the LSS model ($P = 0.42$). Furthermore, using the LSS-derived AUC(0,24 h) for calculating the renal clearance of metformin showed the same trends as derived by Christensen et al. [18] for the gene–gene interaction between OCT2 c.808 (G > T) and MATE1 g.-66 T > C, based on the best-estimated AUC(0,24 h) (Table 3).

Using the LSS model to predict the AUC extrapolated to infinity (AUC(0,∞))

The AUC(0,24 h) of metformin in the development cohort represented 96.8% (95% CI 96.3, 97.4) of the AUC(0,∞) obtained by extrapolation, based on the terminal elimination rate constant. Accordingly, we anticipated that the LSS model validated for the AUC(0,24 h) would provide accurate estimates of the AUC(0,∞). This was verified in the development cohort as well as in the Brazilian cohorts treated with 500 or 1000 mg metformin (Table 4).

Discussion

In the present study we developed and validated limited sampling models that accurately predict the systemic exposure to metformin, assessed by the pharmacokinetic parameter, AUC(0,24 h). LSS development, based on data from healthy Danish adults receiving a single 500 mg oral dose, generated two-point (3 h and 10 h) models, with and without an intercept term, which predicted the individual AUC (0,24 h) with nearly identical accuracy. The statistical principle of parsimony advises in favour of models with fewer

Figure 3

Scatter plot of the relationship between the best estimated AUC (0,24 h) for metformin in 16 previously published studies [23–38] and the corresponding AUC(0,24 h) derived from the two point LSS model (3 h, 10 h, no intercept; Table 1). The best estimated and the LSS-derived AUC(0,24 h)s were obtained as described in Methods. Each study is identified by a distinct symbol. The continuous line is the identity line

Table 2

Impact of OCT2 c.808 (G > T) on the AUC(0,24 h) of metformin

AUC(0,24 h) (µg l^{-1} h)	OCT2 c.808 (G > T) genotype			ANOVA P-value
	GG ($n = 24$)	GT ($n = 18$)	TT ($n = 4$)	
LSS estimated	7.58 (2.9, 11.33)	7.52 (3.79, 12.91)	5.80 (4.82, 6.67)	0.42
Best estimated	7.39 (3.04, 10.55)	7.38 (3.99, 13.48)	5.87 (5.13, 7.01)	0.49

Data from Christensen et al. [18] for 46 healthy subjects using a single dose of 500 mg metformin. Data presented as medians (25th – 75th percentiles). ANOVA P-values for the association of the OCT2 c.808(G > T) genotype with metformin AUC(0,24 h) predicted by the two points LSS AUC (0,24 h) = 4.779*C_3 + 13.174*C_{10} or calculated using all plasma concentration data points (best estimated).

Table 3

Interaction of *OCT2 c.808* (*G > T*) and *MATE1 c.-66* (*T > C*) on the renal clearance of metformin

MATE1 6.-66 T > C	OCT2 c.808 (G > T)			P
	GG	GT	TT	
	Mean CL_{renal} (l h^{-1}) using best-estimated AUC			
TT	27.9 (24.2, 32.2; 7)	32.4 (27.8, 37.8; 6)	44.8 (34.3, 58.4; 2)	0.006
TC	30.8 (27.5, 34.5; 11)	28.0 (24.3, 32.3; 7)	25.5 (19.5, 33.2; 2)	0.202
CC	33.1 (28.0, 39.1; 5)	32.4 (27.4, 38.4; 5)	—	0.275
P	0.148	0.070	0.012	
	Mean CL_{renal} (l h^{-1}) using LSS-derived			
TT	27.5 (23.5, 32.3; 7)	32.5 (27.4, 38.6; 6)	45.4 (33.8, 61.1; 2)	0.013
TC	31.9 (28.15, 36.2; 11)	29.5 (25.1, 34.5; 7)	27.9 (20.7, 37.5; 2)	0.516
CC	32.8 (27.2, 39.6; 5)	31.8 (26.4, 38.4; 5)	—	0.635
P	0.158	0.424	0.090	

Data from Christensen *et al.* [18] for 45 healthy subjects using a single dose of 500 mg metformin. Data are presented as geometric means (95% CI; number of individuals). CL_{renal} renal clearance. *P* values refer to parametric multiple regression analysis, adjusted for the individual glomerular filtration rates, for the interaction of the *OCT2 c.808*(*G > T*) and *MATE1 g.-66 T > C* genotypes on metformin CL_{renal} calculated using the LSS-derived AUC (AUC(0,24 h) = 4.779*C_3 + 13.174*C_{10}) or using all plasma concentration data points, as reported by Christensen *et al.* [18].

parameters, and the two-point model without intercept was chosen for validation in independent cohorts. These cohorts comprised male and female individuals of various ethnicities, healthy volunteers, patients with diabetes, polycystic ovarian syndrome and post-gastric bypass surgery, a wide range of metformin doses (250–3000 mg day^{-1}) and various formulations. Collectively, the validation tests confirmed the accuracy and precision of the AUC(0,24 h) estimates provided by the LSS algorithm based on two plasma concentrations (3 h and 10 h after oral drug intake) with no intercept. This LSS model was also shown to predict accurately the AUC(0,∞) in the development cohort as well in the validation cohorts of healthy Brazilians administered 500 or 1000 mg doses of metformin.

We suggest that the validated LSS algorithm is appropriate for predicting the systemic exposure to metformin after single or chronic administration and may be used to replace non-compartmental analysis in pharmacokinetic studies,

including pharmacogenetic trials. Accordingly, we showed that the two point LSS equation led to the same conclusions, as those derived from traditional non-compartmental methods [18], regarding both the effect of a polymorphism in the OCT2 transporter (*OCT2 c.808G > C*) on metformin AUC(0,24 h) and the influence of gene-gene interaction between *OCT2* c.808 (*G > T*) and *MATE1 g.-66 T > C* on the renal clearance of metformin.

Although plasma concentrations of metformin are not monitored in clinical practice, this may be useful, particularly in patients with renal impairement, in order to ensure that a safe dose is being administered [39]. Graham *et al.* suggested that the average concentration of metformin in plasma at steady-state over a dosage interval ($C_{av,ss}$) provides the best correlates with the clinical effects of metformin. Since $C_{av,ss}$ may be derived directly from the AUC for the respective dosage interval, our validated LSS model may prove useful for studies exploring the tentative recommendation of a

Table 4

r^2, bias and precision of the LSS model to the predict the AUC(0,∞) of metformin

Cohorts	r^2	MD (%) mean (95% CI)	MAD (%) mean (95% CI)
Development cohort			
Healthy Danes, single dose, 500 mg	0.919	−3.7 (−5.9, 1.1)	7.3 (5.7, 8.8)
Validation cohorts			
Healthy Brazilians, single dose, 500 mg	0.878	0.4 (−6.0, 6.8)	8.7 (2.4, 15.0)
Healthy Brazilians, single dose, 1000 mg	0.921	−1.0 (−7.9, 5.9)	7.8 (1.8, 13.8)

LSS model equation: AUC(0,24 h) = 4.779*C_3 + 13.174*C_{10}. r^2 correlation coefficient between LSS-predicted AUC(0,24 h) and the extrapolated AUC (0,∞); MD bias; MAD precision of the LSS estimates.

maximal value of $C_{av,ss}$ of 2.5 mg l^{-1}, to prevent toxicity due to lactic acidosis [39]. In this regard, the lack of validation of the LSS model in patients with moderate or severe renal impairment, who are at higher risk for metformin toxicity, may be seen as a limitation of our study.

To our knowledge, only one published study has previously explored metformin pharmacokinetic parameters, using the LSS approach. Chen et al. [25] applied the procedures described by Suarez-Kurtz et al. [13, 14] to a bioequivalence trial in a cohort of 20 healthy volunteers and derived a two point (4 h and 10 h) equation with intercept, which provided good estimates of the AUC(0,24 h) ($r^2 = 0.94$) for the 1000 mg dose of the metformin reference formulation. This LSS model was validated using data from the same cohort and, not surprisingly, was found accurate. When applied to the cohorts of our study treated with single metformin doses (Supplementary Table S1), the model developed by Chen et al. [25] compared unfavourably with our two point LSS algorithm (Table 1) regarding bias and precision and, to a smaller extent, r^2. For example, in Brazilians receiving a single 1000 mg dose, the bias and precision of the LSS estimates were 1.0 (−0.3–3.3), and 7.2 (6.3–8.1) for our two point algorithm with no intercept, compared with 7.9 (6.5–9.3) and 10.5 (9.5–11.5) for the algorithm described by Chen et al. [25].

In conclusion, we show that two point LSS models allow accurate estimation of metformin's AUC(0,24 h) under a wide variety of demographical, clinical and experimental conditions, metformin doses, formulations and frequency of administration. The small number ($n = 2$) of samples required for the model represent considerable reduction of cost of analysis and save time both for subjects in trials and investigators. Using the LSS model we reproduced the results reported by Christensen et al. [18] for the effects of polymorphisms in the *OCT2* and *MATE1* genes on AUC (0,24 h) and renal clearance of metformin. This suggests a potential usefulness of our LSS models in pharmacogenetic trials and we look forward to additional analyses, retrospective or prospective, to verify this notion.

Competing Interests

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Supporting Information

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Figure S1 Bland–Altman plot of the difference between the best-estimated and LSS-derived AUC(0,24 h)'s of metformin, against the best-estimated AUC(0,24 h), in the development cohort. The LSS estimates were obtained using the two point LSS models with or without an intercept term, presented in the text and Table 1. Black circles and continuous lines refer to the LSS model without intercept; open circles and dashed lines refer to the LSS model with intercept. The horizontal lines are drawn at the mean difference and at + 1.96 sds of the mean difference between best-estimated and LSS-derived AUC(0,24 h) of metformin

Figure S2 Loess smooth curve fitting to the correlation between the best-estimated and LSS-predicted AUC(0,24 h)'s of metformin in the development cohort. The LSS estimates were obtained using the twopoint LSS models with or without an intercept term, presented in the text and Table 1. Black circles and thick continuous line refer to the LSS model without intercept, open circles and dashed line refer to the LSS model with intercept. The thin straight line represents the identity line

Figure S3 Bland–Altman plot of the difference between best-estimated and LSS-derived AUC(0,24 h) of metformin, against the best-estimated AUC(0,24 h), in the validation cohort of healthy Brazilians receiving a single 500 mg dose of metformin. The LSS estimates were obtained using the 2-point LSS model without intercept. Each individual is represented by a black circle. The horizontal lines are drawn at the mean difference and at + 1.96 sds of the mean difference between best-estimated and LSS-derived AUC(0,24 h) of metformin

Figure S4 Loess smooth curve fitting to the correlation between best-estimated and LSS-predicted AUC(0,24 h) of metformin in the validation cohort of healthy Brazilians receiving a single 500 mg dose of metformin. The LSS estimates were obtained using the two point LSS model without intercept. Each individual is represented by a black circle. The thin straight line represents the identity line

Figure S5 Bland–Altman plot of the difference between best-estimated and LSS-derived AUC(0,24 h) of metformin, against the best-estimated AUC(0,24 h), in the validation cohort of healthy Brazilians receiving a single 1,000 mg dose of metformin. The LSS estimates were obtained using the two point LSS model without intercept. Each individual is represented by a black circle. The horizontal lines are drawn at the mean difference and at + 1.96 sds of the mean difference between best-estimated and LSS-derived AUC(0,24 h) of metformin

Figure S6 Loess smooth curve fitting to the correlation between best-estimated and LSS-predicted AUC(0,24 h) of metformin in the validation cohort of healthy Brazilians receiving a single 1000 mg dose of metformin. The LSS estimates were obtained using the two point LSS model without intercept. Each individual is represented by a black circle. The thin straight line represents the identity line

Figure S7 Bland–Altman plot of the difference between best-estimated and LSS-derived AUC(0,24 h) of metformin, against the best-estimated AUC(0,24 h), in 16 previously published studies . The LSS estimates were obtained using the two point LSS model without intercept. Each study is represented by a black circle. The horizontal lines are drawn at the mean difference and at + 1.96 sds of the mean difference between best-estimated and LSS-derived AUC(0,24 h) of metformin

Figure S8 Loess smooth curve fitting to the correlation between best-estimated and LSS-predicted AUC(0,24 h) of metformin in 16 previously published studies . The LSS estimates were obtained using the two point LSS model without intercept. Each study is represented by a black circle. The thin straight line represents the identity line

Table S1 Validation of the LSS algorithm described by Chen *et al.* [25]